Auris Nasus Larynx 39 (2012) 369–373

Contents lists available at SciVerse ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Frequency of *Alloicoccus otitidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* in children with otitis media with effusion (OME) in Iranian patients

Seyed Sajjad Khoramrooz [a], Akbar Mirsalehian [a,*], Mohammad Emaneini [a], Fereshteh Jabalameli [a], Marzieh Aligholi [a], Babak Saedi [b], Abdollah Bazargani [c], Morovat Taherikalani [d], Pedram Borghaei [e], Ebrahim Razmpa [b]

[a] *Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[b] *Otorhinolaryngology Research Centre, Department of Otolaryngology – Head and Neck Surgery, Imam Khomeini Complex Hospital, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Bacteriology and Virology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran*
[d] *Department of Microbiology, School of Medicine, Ilam University of Medical Sciences, Ilam, Iran*
[e] *Department of Otolaryngology – Head and Neck Surgery, Amir-A'lam Hospital, Tehran University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 28 April 2011
Received in revised form 29 June 2011
Accepted 4 July 2011
Available online 25 August 2011

Keywords:
Otitis media with effusion
Alloicoccus otitidis
Streptococcus pneumoniae
Moraxella catarrhalis
Haemophilus influenzae

ABSTRACT

Objective: To determine the presence of common bacterial agents of otitis media with effusion (OME), together with investigation these agent in the adenoid tissue and antimicrobial susceptibility pattern of isolated bacteria in Iranian children with OME.
Methods: Polymerase chain reaction (PCR) and bacterial culture methods were used for detection and isolation of *Alloicoccus otitidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* in 63 middle ear fluid samples and 48 adenoid tissues from 48 OME patients. Fifteen patients were bilaterally affected. Antimicrobial susceptibility of all bacterial isolates were determined by disk agar diffusion (DAD) method.
Results: Bacteria were isolated from 47% (n = 30) of the middle ear fluid samples and 79% (n = 38) of the adenoid tissue specimens in OME patients. *A. otitidis* was the most common bacterial isolated from the middle ear fluid 23.8% by culture and 36.5% by PCR method. *S. pneumoniae* was the most prevalent pathogen (35.5% and 31.2% by culture and PCR) in the adenoid tissues. In 10 patients the same organisms were isolated from the middle ear fluid and adenoid tissue. Antimicrobial susceptibility pattern showed taht most isolates of bacteria were sensitive to ampicillin, Amoxicillin/Clavulanate and fluoroquinolones.
Conclusion: The present study, being the first report on the isolation of *A. otitidis* by culture method in Iran and Asian countries, shows that *A. otitidis* is the most frequently isolated bacterium in Iranian children having otitis media with effusion. In this study *A. otitidis*, *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* are the major bacterial pathogens in patients with OME and we found that ampicillin and Amoxicillin/Clavulanate have the excellent activity against bacterial agents in Iranian children with OME.

1. Introduction

Otitis media is one of the most common childhood diseases and it is the main cause of several otological problems [1]. Otitis media with effusion (OME) and acute otitis media (AOM) are the two major sub-classifications of otitis media [2]. OME is characterized by the presence of middle ear fluid without acute infection [3] and its prevalence among children is approximately 20% [4]. The peak incidence of OME occurs at the first year of age [5]. Two weeks after the beginning of otitis media, about 70% of children have fluid in the middle ear and after one month it is decreased to 40%. Within 3 months after the first signs of infection still 10% of the children have fluid in the middle ear [6]. The effect of OME on hearing loss [3] also has a negative effect on the development of language in the first 3 years of age [7]. Although the etiology of OME is still unclear, bacterial and viral infections have an important role in its pathogenesis [8]. It has been shown that bacterial agents are present in 22–52% of OME cases [9]. *Haemophilus influenzae*,

* Corresponding author at: Microbiology Department, School of Medicine, Tehran University of Medical Sciences, 100 Poursina St., Keshavarz Blvd., Tehran, Iran. Tel.: +98 21 8895 5810; fax: +98 21 8895 5810.
E-mail address: mirsaleh@sina.tums.ac.ir (A. Mirsalehian).

0385-8146/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.anl.2011.07.002

Streptococcus pneumoniae and *Moraxella catarrhalis*, isolated by culture, are the predominant bacterial pathogens of OME [10]. *Alloicoccus otitidis* (formerly *Alloicoccus otitis*) isolated for the first time by Faden and Dryja [11] and then by biochemical test and genetic analysis, was proposed as a new genus [12] and later; its name was revised to *A. otitidis* [13]. *A. otitidis* is a slow growing and fastidious organism, so it is difficult to isolate this microorganism by conventional culture [14]. However by PCR, *A. otitidis* was detected in about 18.5–60.5% of OME patients, which was more than the cases detected for the three major pathogens [15]. Some studies have suggested the association of otitis media with chronic adenoidal infection, through the transmission of bacteria from adenoidal infection via the Eustachian tubes into the middle ear [16,17]. It is demonstrated that adenoid tissue has an important role in the pathophysiology of OM [16]. Paradise et al. reported the role of adenoidectomy in the treatment of children with recurrent OME who have previously had tympanostomy tubes placement [18]. It was shown that pathogenic bacteria are more frequently isolated from the adenoid tissue of patients with recurrent or persistent otitis, compared with patients with adenoid hypertrophy alone [19]. Karlidag et al. demonstrated a similarity between isolated bacteria in the middle ear effusion and the cultured bacteria in the adenoid tissue [20]. There is no information about the etiology of bacterial agents in children with OME in Iran. The isolation of *A. otitidis*, one of the major agents of OME, has also not been reported in Asian countries. The aim of the current study was, therefore, to determine the common bacterial agents and their susceptibility pattern among patients with OME.

2. Materials and methods

2.1. Patients and sample collection

Two kinds of clinical samples were collected: Middle ear fluid ($n = 63$) and adenoid tissue ($n = 48$) obtained from 48 patients with persistent middle ear effusion without symptoms of acute otitis media. Fifteen patients were bilaterally affected whereas 33 children had unilateral disease, hence both right and left middle ear fluid were collected. Collected samples were from patients who attended the Department of Otolaryngology of Imam Khomeini and Amir Alam, two teaching hospitals in Tehran University of Medical Sciences, during September 2009–November 2010.

Inclusion criteria for myringotomy and insertion of a ventilation tube in OME patients were the presence of middle ear effusion for more than 3 months and not being on antibiotic therapy 2 weeks before and at the time of the surgery. In addition, all of these patients had adenoid hypertrophy and they were candidate for adenoidectomy. Children with previous transtympanic ventilation tubes, tympanic membrane perforations, previous adenoidectomy, immunological defect, anatomic abnormality, respiratory tract infection and purulent middle ear fluid were excluded.

Before surgical procedure and specimen collection, the external ear canal was disinfected with povidone-Iodine for 2 min and then washed three times with sterile normal saline for eliminating the antiseptic agent. After myringotomy, middle ear fluid was aspirated into a Juhn-Tym-Tap collector (Xomed Inc., Jacksonville, USA). In bilateral cases, both middle ear fluid of the right and left ears were collected. The adenoid tissue samples obtained after adenoidectomy were placed in a sterile container. All of the clinical samples were sent to the Laboratory of Microbiology Department within two hours. Prior to sample collection, written informed consents were obtained from parents of each individual. The present study was approved by the University Ethics Committee. Past medical histories and demographic data of patients were collected from their medical records, prior to surgery.

2.2. Isolation of bacteria

Each specimen was divided into two portions; one for culture and the other for Multiplex PCR assay. For primary isolation of bacteria, specimens were inoculated to several culture media under aerobic conditions with 5% CO_2 at 35 °C for 24–72 h according to Chapin, Vaneechoutte and Bosley methods [21–23]. The culture medias were as follows: Muller Hinton with 5% Sheep blood agar (for *S. pneumoniae* and *A. otitidis*), chocolate agar with vancomycin (5 μg/ml), clindamycin (1 μg/ml) and bacitracin (300 μg/ml) for *H. influenzae* [21], chocolate agar with vancomycin (5 μg/ml), clindamycin (1 μg/ml) and bacitracin (300 μg/ml) and acetazolamide (for *M. catarrhalis*) [22]. Because of the slow growth of *A. otitidis*, incubation was extended to 14 days [23]. Isolated bacteria were identified by conventional biochemical methods [24].

2.3. Multiplex-PCR

Bacterial DNA was extracted using a RTP® Bacteria DNA Mini Kit (Invitek GmbH, Berlin, and Germany) according to the manufacturer's instructions. DNA was extracted from adenoid tissue according to Bartlett and Stirling method [25]. Multiplex PCR amplification of 16S rRNA genes was done with specific primers according to Hendolin et al. method [26]. Polymerase chain reaction was carried out to a total volume of 50 μl containing 5 μl of DNA extracted from bacterial suspensions or adenoid tissues, 5 pmol of each primer, 1× PCR buffer, 5 mM $MgCl_2$, 100 μl each of the deoxy-nucleotide triphos phate (Fermentas) and 1.5 U of Taq polymerase (Fermentas). PCR conditions were as follows: an initial denaturing at 94 °C for 5 min, followed by 35 cycles denaturing at 94 °C for 30 s, annealing at 60 °C for 45 s and extension at 72 °C for 40 s. The final extension was continued at 72 °C for 5 min. The multiplex PCR products were detected by electrophoresis of 20 μl of each amplification mixture in 1.5% agarose gels. Gels were stained with ethidium bromide and then visualized by UV light illumination Fig. 1. For confirmation of *A. otitidis*, sequences of 16S rRNA of all isolates were aligned in NCBI.

2.4. Antimicrobial sensitivity

The disk agar diffusion (DAD) method was used to determine the susceptibility patterns among the strains, according to the CLSI (Clinical and Laboratory Standards Institute) guidelines [27].

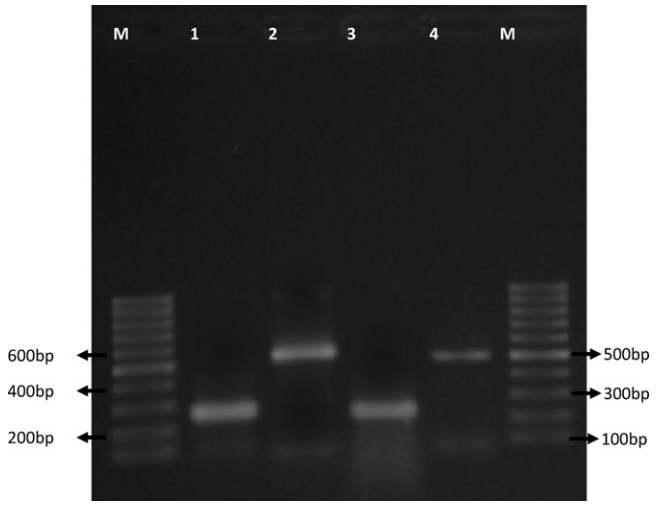

Fig. 1. Detection of *A. otitidis*, *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* by multiplex PCR. The sizes of PCR products are: 1- *A. otitidis* (264 bp), 2- *H. influenzae* (525 bp) 3- *M. catarrhalis* (237 bp) and 4- *S. pneumoniae* (484 bp). M stands for molecular size marker.